Association of the *bla*$_{CMY-10}$ gene with a novel complex class 1 integron carrying an IS*CR1* element in clinical isolates from Korea

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Abstract

The *bla*$_{CMY-10}$ gene responsible for β-lactam resistance was located on a new complex class 1 integron within a conjugative plasmid. The *sul1*-type class 1 integron, containing an *aadA2a* gene cassette, was identified upstream of *bla*$_{CMY-10}$. A unique gene array (*yqgF–yqgE–gshB–orf97–orf105*) was identified downstream of *bla*$_{CMY-10}$.

Keywords: AmpC, *bla*$_{CMY-10}$, complex class 1 integron, *gshB*, IS*CR1*

Original Submission: 4 April 2009; **Revised Submission:** 8 July 2009; **Accepted:** 9 July 2009
Editor: P. Tassios
Article published online: 22 October 2009

Clin Microbiol Infect 2010; **16:** 1013–1017
10.1111/j.1469-0691.2009.03002.x

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Resistance to β-lactam antibiotics in Gram-negative bacteria is mainly mediated by the production of β-lactamases, which are divided into four major Ambler molecular classes (A, B, C, and D). In previous studies, we have demonstrated that CMY-10 from a virulent clinical strain is a plasmid-encoded extended-spectrum class C (AmpC) β-lactamase conferring resistance to cefoxitin and cefotetan as well as to penicillins and oxyimino-cephalosporins [1]. Furthermore, our structural analyses led us to demonstrate that the widening of the active site by the deletion of some residues in the R2-loop can be considered as an operative molecular strategy of class C β-lactamases to extend their substrate spectrum to oxyimino-cephalosporins and carbapenems [2]. We previously obtained six clinical isolates resistant to β-lactams, including cefoxitin, and characterized them as CMY-10 producers [3]. A *bla*$_{CMY-10}$ gene, responsible for this β-lactam resistance, was located on a 130-kbp conjugative plasmid harboured by six clinical isolates and their transconjugants [3]. Clonal and plasmid (mainly) spread contributed to *bla*$_{CMY-10}$ dissemination among members of the family *Enterobacteriaceae* in Korea [3]. This study was carried out to investigate the genetic environment surrounding the *bla*$_{CMY-10}$ gene, which can play an essential role in the dissemination of the gene.

Replicon typing of the *bla*$_{CMY-10}$-carrying plasmid was performed by a PCR method based on replicons (inc/rep-PCR) of the major plasmid incompatibility groups among the *Enterobacteriaceae* [4]. The replicon type of the conjugative plasmid was HI2, which was reported to be predominant among *Enterobacteriaceae* in Korea until 1998 [5]. PCR and DNA sequence analyses (using the primers in Table 1) of six clinical isolates revealed that *bla*$_{CMY-10}$ was located in a new complex class 1 integron within the conjugative plasmid (Fig. 1a). To characterize this integron, recombinant plasmids with overlapping fragments were obtained as follows. Long and accurate PCR amplification was performed with specific primers (Table 1). The long and accurate PCR products were ligated with the pCR2.1-TOPO cloning vector (Invitrogen). The ligation mixture (recombinant plasmids) was introduced into competent *Escherichia coli* DH5α cells (Invitrogen) by transformation; kanamycin (50 mg/L; Sigma-Aldrich) was used for transformant selection. In Fig. 1b, the recombinant plasmids are pTO21-47.1A (3139 bp; a recombinant plasmid carrying the *sul1*-type integron), pTO21-47.1B (3752 bp; a recombinant plasmid carrying a region from within *sul1* to within *bla*$_{CMY-10}$), and pTO21-47.1C (5036 bp; a recombinant plasmid carrying a region from within *bla*$_{CMY-10}$ to *qacE*Δ*1*(*140*)/*sul1*). Consequently, the plasmids covered, together, a 10 925-bp region containing *bla*$_{CMY-10}$. The sequence of this complex class 1 integron has been sub-

TABLE 1. Primer sequences used in this study

Target and primer[a]	Sequence	Expected PCR product (bp)
bla$_{CMY-10}$		
C1	5′-GAG CAG ACC CTG TTC GAG AT-3′	847
C2	5′-GAT TGG CCA GCA TGA CGA TG-3′	
sul1		
sulf-F	5′-ATG GTG ACG GTG TTC GGC AT-3′	840
sulf-R	5′-CTA GGC ATG ATC TAA CCC TCG GTC-3′	
sul1-F	5′-GAT TTT TCT TG AGC CCC GC-3′	155
sul1-R	5′-TGG ACC CAG ATC CTT TAC AGG-3′	
qacEΔ1		
qacEΔ1-F	5′-GTT ATC GCA ATA GTT GGC G-3′	227
qacEΔ1-R	5′-AGC TTT TGC CCA TGA AGC AAC C-3′	
intI1		
intIf-F	5′-CTA CCT CTC ACT AGT GAG GGG CG-3′	1014
intIf-R	5′-ATG AAA ACC GCC ACT GCG C-3′	
intI1-F	5′-CCT CCC GCA CGA TGA TCG TGC-3′	280
intI1-R	5′-TCC ACG CAT CGT CAG GCA TTG-3′	
IS*CR1*		
orf513-1F	5′-ATG TCG CTG GCA AGG AAC GC-3′	1538
orf513-2R	5′-TCA AAG AGA CGA CTC TGT GAT GGA TC-3′	
orf513-3F	5′-TGA CTC TTA TCC AAC GCT TTG GC-3′	480
orf513-4R	5′-CTG GCC GAC TAA TGT AGC GAC AC-3′	
aadA2a		
2-int-1-F	5′-AAG TTA GAC ATC ATG AGG GTA-3′	727
2a-sul1-w2-F	5′-AGT GAT CTT CTT TTT GTC CCA-3′	
IS*CR1*, *bla*$_{CMY-10}$		
2-qac-w2-F	5′-CAA ACA GAC GAG AAA CAG CCC CAA-3′	1948
2-CMY-10-1-R	5′-ATG AAT CCA CCT CCT CGG G-3′	
2-qac-w3-F	5′-TGC TCA GCT TTC CTT TCC AGC TAC G-3′	2034
2-CMY-10-w2-R	5′-AAT TCC CTC ACT CGT TTA CCG CTC-3′	
*yqg*F, *yqg*E, *gsh*B		
2-CMY-10-1-F	5′-CAA TTC GGC CAA GGT GAT C-3′	2250
2-sul1-w3-R	5′-ACC GAG TTC ATC TAC GCC ATC TAC A-3′	
*yqg*E, *gsh*B, *orf97*		
2-CMY-10-w2-F	5′-CTG CGT GAT GAC ATG GGG TTC CTT A-3′	2163
2c-sul1-w2-R	5′-CGC CCT ACA GCA TCA ACA CCC T-3′	
*gsh*B, *orf97*, *orf105*		
2-CMY-10-w3-F	5′-GCT TGA GCT TGG TCA GCA TGG TGT C-3′	2270
2-sul1-1-R	5′-CAG AAT GCC GAA CAC CGT CAC-3′	

[a]Orientation of each primer: F, forward; R, reverse.
The BLASTN (Basic Local Alignment Search Tool against nucleotide sequence database) program of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) was used for DNA sequence database searches of integron element genes and other genes. The primers for PCR amplification were designed by selecting consensus sequences in multiple nucleotide alignments (CLUSTAL W program) of detected genes.

mitted to the GenBank database under accession no. FJ004895.

A *sul1*-type class 1 integron containing an *aadA2a* gene cassette was identified upstream of *bla*$_{CMY-10}$ and ended with a 3′ conserved segment (*qacEΔ1-sul1*). The following 2.15 kbp showed high homology with a region carrying an IS*CR1* element (previously designated *orf513* or *orf341*) in In6 of pSa [6] and integrons of pAr-32 [7], pSAL-1 [8], pAJE0508 [9], pCMXR1 [10], and pKO56 [11]. Recently, it has been suggested that IS*CR1* is a member of an extended family of IS*91*-like elements that can transpose adjacent DNA sequences by a mechanism termed rolling-circle transposition and are responsible for the mobilization of many classes of antibiotic resistance genes, including *bla*$_{CMY-9}$, *bla*$_{DHA-1}$, *bla*$_{CTX-M-14}$, *catA2*, and *qnr*, but not *bla*$_{CMY-10}$ thus far [12]. A recombination cross-over site (RCS) (33-bp DNA sequence containing *ori*IS) at which insertion of resistance genes into the complex class 1 integron containing IS*CR1* takes place was observed downstream of IS*CR1* [13]. A 179-bp region identified between the RCS and the start codon of *bla*$_{CMY-10}$ is closely related to the region of pMVP-1 (92% identity) containing *bla*$_{CMY-1}$, the region of pK29 (63% identity) containing *bla*$_{CMY-8}$ [14], and the region of pCMXR1 (63% identity) containing *bla*$_{CMY-9}$ (Fig. 1c). In other words, the 179-bp region downstream of the 3′-end of RCS revealed that, as compared with the upstream sequence of *bla*$_{CMY-1}$ (GenBank accession no. X92508), the upstream sequence of *bla*$_{CMY-10}$ had an additional 15 bases. On the other hand, the upstream sequences of *bla*$_{CMY-9}$ [10] and *bla*$_{CMY-8}$ [14] lacked 50 bases when compared with the upstream sequence of *bla*$_{CMY-1}$. These nucleotide sequence analyses of *bla*$_{CMY-1}$, *bla*$_{CMY-8}$, *bla*$_{CMY-9}$ and *bla*$_{CMY-10}$ indicate

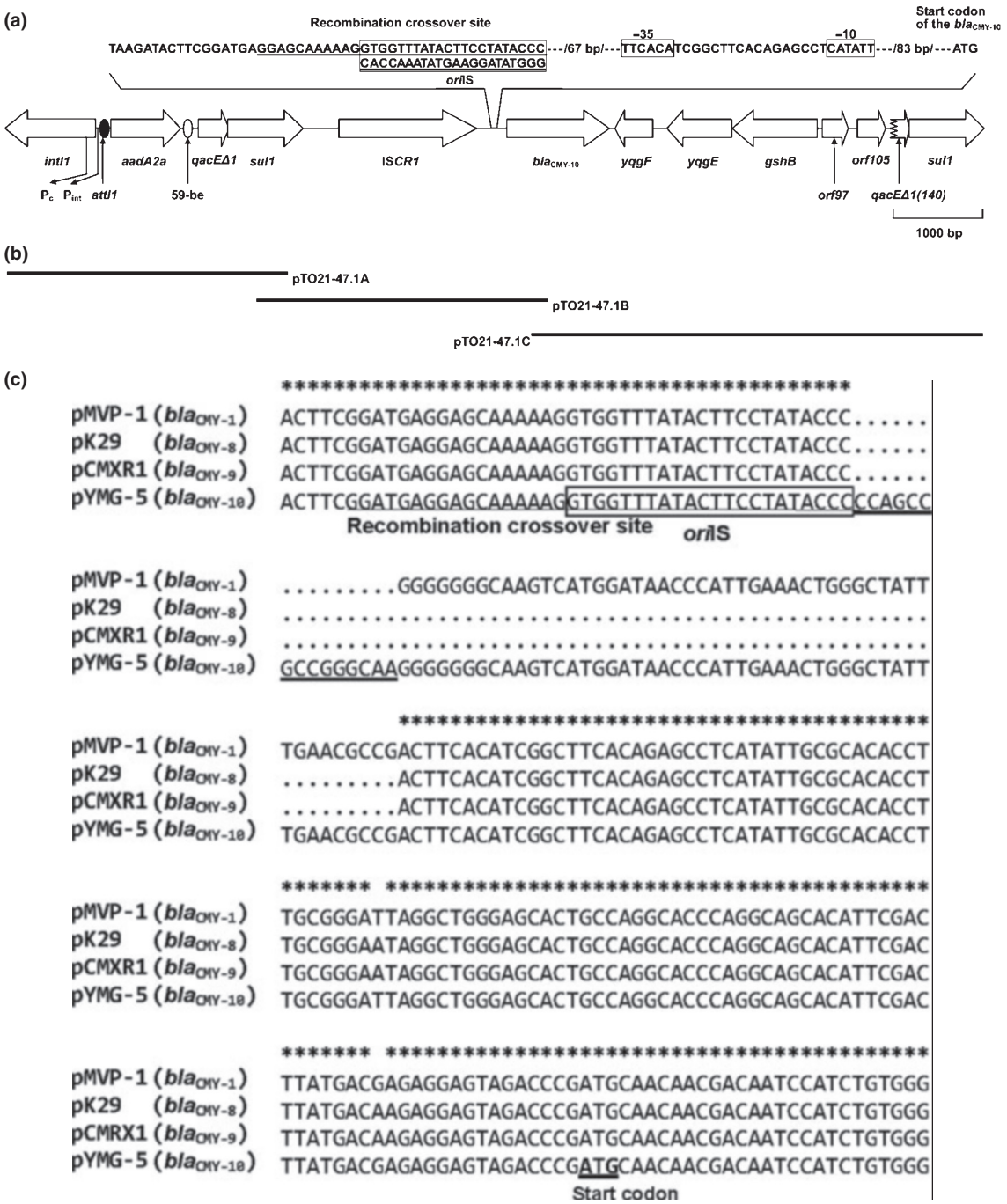

FIG. 1. (a) Schematic representation of the backbone structure of the complex class 1 integron harbouring novel genes on a 130-kbp conjugative plasmid from an *Enterobacter aerogenes* isolate. Overlapping PCR amplifications and DNA sequencing with specific primers (Table 1) revealed that the new integron was identical in the remaining clinical isolates. Open arrow, open reading frames; white oval, 59-be element; black oval, *attI1* recombination site; P_c (5′-TGGACATAAGCCTGTTCGGTTCGTAAACT-3′) and P_{int} (5′-AGTCTATGCCTCGGGCATCCAAGCAGCAA-3′) are promoters for cassette genes and the integrase (*intI1*) gene, respectively. The −35 and −10 motifs of the bla_{CMY-10} gene promoter are boxed. The recombination cross-over site is underlined. *ori*S (the initiation site of IS*CR1* transposition) is boxed and underlined. *qacEΔ1*(*140*) shows a 140-bp deletion at the 5′-end of *qacEΔ1*, and the remainder of the two genes are identical. (b) The three subclones used for sequencing. (c) Comparison of the upstream nucleotide sequences of four plasmid-mediated bla_{CMY} β-lactamase genes. Sequence identity is indicated by asterisks. The absence of some nucleotides is indicated by dots. The recombination cross-over site (RCS) and *ori*S are underlined and boxed, respectively. The additional 15 bases described in this study are heavily underlined. The start codon of the bla_{CMY} genes is bold and heavily underlined. GenBank accession numbers are as follows: bla_{CMY-1}, X92508; bla_{CMY-8}, EF382672; bla_{CMY-9}, AB061794; bla_{CMY-10}, FJ004895.

that there might be past transposition events medicated by the IS*CR1* element upstream of bla_{CMY-10} because of different genetic environments downstream of the 3′-end of the RCS in these genes. An earlier report has suggested that this variation is indicative of past transposition events [10]. These results indicate that the IS*CR1* element can mobilize bla_{CMY-10} into mobile plasmids and so promote its dissemination among *Enterobacteriaceae*. Since the first emergence of bla_{CMY-10}, [3], the gene has continuously spread among *Enterobacteriaceae* (four *E. coli* and two *Klebsiella pneumoniae* in 2003 [15], two *Proteus mirabilis* in 2004 [16], and two *E. coli* in 2005 [17]) in Korea.

A unique gene array (*yqgF–yqgE–gshB–orf97–orf105*) was first identified between bla_{CMY-10} and a duplication (*qacEΔ1(140)-sul1*) of the 3′ conserved segment (Fig. 1a). The third open reading frame (*gshB*) encoded a 316-amino-acid protein that shared 95% amino acid identity with the amino acid sequence of glutathione synthetase (GshB) of *Aeromonas hydrophila* [18]. The nucleotide sequence identity of the unique gene array with chromosomal segments (AHA-3127, AHA-3128, AHA-3130 and AHA-3131 in GenBank accession no. CP000462) from *A. hydrophila* was more than 83%, suggesting that this unique gene array may have originated in the chromosomal DNA of *A. hydrophila*.

As compared with nucleotide sequences of other complex class 1 integrons, the region *intI1–aadA2a–qacEΔ1–sul1*–IS*CR1*–RCS in the new integron shares 99% identity with those of In6 and integrons of pAr-32, pSAL-1 and pAJE0508 detected in Australia, Norway, France, and Korea, respectively [7–9]. The region bla_{CMY-10}*–yqgF–yqgF* between the RSC and *gshB* shares 98% identity with those of integrons associated with pCMXR1 and pK29 [14] from Japan and Taiwan. The region *orf105–qacEΔ1(140)–sul1* in the integron shares 99% identity with that of the integron of pKO56 from an isolate recovered in Korea [11]. These results suggest that the integron containing bla_{CMY-10} might be derived from these integrons identified predominantly in East Asia.

Acknowledgements

We would like to thank S. G. Kang for critical reading of the manuscript and valuable comments.

Transparency Declaration

This work was supported by research grants from the Korea Research Foundation (KRF-2008-313-C00790), the Korea Science and Engineering Foundation (KOSEF), funded by the Korean Government (MEST) (2009-0077922), the Driving Force Project for the Next Generation of Gyeonggi Provincial Government in Korea, and the Marine & Extreme Genome Research Centre Program of Ministry of Land, Transport, and Maritime Affairs in Korea. The funding agencies had no role in the design or conduct of the study, analysis or interpretation of the data, or preparation, review or approval of the manuscript. The authors declare no conflict of interest concerning the funding agencies or a commercial company.

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Genetic diversity in CC398 methicillin-resistant *Staphylococcus aureus* isolates of different geographical origin

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Abstract

Staphylococcus aureus of sequence type 398 has emerged in Europe, North America and Asia, and has typically been associated with livestock and their human contacts. We analysed two Panton–Valentine leukocidin (PVL)-negative t034-ST398 isolates from humans in contact with pigs and two t034-ST398 PVL-positive isolates from two unrelated, adopted Chinese children, using multistrain microarrays to determine genomic variability between the two sets of isolates. The ST398 isolates clearly belong to the same lineage when compared to other clonal lineages. However, the four isolates cluster into two distinct groups corresponding to differences in epidemiology based on mobile genetic elements and resistance patterns, suggesting that the two groups are epidemiologically distinct.

Keywords: Methicillin-resistant *Staphylococcus aureus*, mobile genetic elements, multistrain microarray, Panton–Valentine leukocidin, ST398

Original Submission: 1 May 2009; **Revised Submission:** 7 July 2009; **Accepted:** 7 July 2009
Editor: G. Lina
Article published online: 20 October 2009

Clin Microbiol Infect 2010; **16:** 1017–1019
10.1111/j.1469-0691.2009.03003.x

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Staphylococcus aureus has generally been classified as an opportunistic human pathogen causing nosocomial and, in recent years, increasing numbers of community-acquired infections worldwide [1,2]. However, methicillin-resistant *S. aureus* (MRSA) has recently been found also in both pet animals and a variety of livestock animals, such as poultry, cattle and pigs [3,4]. A new clone discovered in 2004, of sequence type (ST) 398, has been found since in several European countries, the USA, Canada, Singapore and China [5,6]. The carriage prevalence of this clone is very high in livestock farmers and veterinary personnel [7]. Molecular analysis has shown isolates belonging to clonal complex (CC) 398, including ST398, to be generally non-typeable by standard *Sma*I pulsed-field gel electrophoresis analysis, due to DNA methylation, and to encode *spa* type t011, *spa* type t034 or close variants thereof, the majority of which carry SCC*mec* V [6,8,9]. This clone has shown a rapid increase in prevalence in The Netherlands [7], as well as in Denmark (manuscript in preparation), and has caused a nosocomial outbreak in The Netherlands [10]. A significant number of successful community-acquired lineages encode Panton–Valentine leukocidin (PVL), which has been associated with severe skin infections and necrotizing pneumonia [11,12]. So far, CC398 isolates from pigs and people in contact with pigs have been identified as PVL-negative. However, PVL-positive CC398 MRSA isolates have recently been described sporadically in China [5] and in Europe [13,19]. As part of a national surveillance of MRSA-positive persons, we identified two PVL-positive MRSA CC398 isolates in two unrelated children adopted from China with skin and soft tissue infections. These two isolates were compared with two isolates of the predominant type found in people with contact with pigs in Denmark by multistrain microarray analysis covering 3623 open reading frames, to estimate the genetic diversity of this epidemic clone.